

REPLACED BY
ART 34 ANDTWe claim

1. A process for the enzymatic hydrolysis of carotenoid esters, where a carotenoid ester-containing reactant derived from a natural or genetically modified organism is incubated with an ester-cleaving enzyme selected from carboxylic ester-cleaving hydrolases (E.C. 3.1.1.) until the hydrolytic ester cleavage is essentially quantitative, and the carotenoid(s) which is(are) formed is(are) isolated where appropriate from the reaction mixture.
2. A process as claimed in claim 1, where the reaction time is in the range from 1 to 48 hours.
3. A process as claimed in claim 1, where the hydrolase is selected from lipases (E.C. 3.1.1.3) and esterases (E.C. 3.1.1.13), and mixtures thereof.
4. A process as claimed in any of the preceding claims, where the total amount of enzyme added to the reaction mixture is such that the total concentration of added enzyme based on the total carotenoid content is in the following range:
 - a) Lipase: 50 to 3 000 U/ μ g
 - b) Esterase: 10 to 500 U/mg
5. A process as claimed in any of the preceding claims, where a carotenoid ester-containing reactant is incubated in an aqueous reaction medium with the ester-cleaving enzyme, with the carotenoid esters being present where appropriate in emulsified form in the medium, and with at least one emulsifier being added where appropriate to the reaction medium in an amount such that the ratio of the amounts of emulsifier to total carotenoid is in the range from 500:1 to 1000:1.
6. A process as claimed in any of the preceding claims, where the emulsifier includes at least one compound selected from cholanolic acid and derivatives thereof, and mixtures of these compounds.
7. A process as claimed in claim 5, where the emulsifier is a mixture of cholic acid and deoxycholic acid.
8. A process as claimed in any of the preceding claims, where the reaction mixture has a pH in the range of about 6 to 8.

9. A process as claimed in any of the preceding claims, where the reaction medium is an aqueous/organic medium which comprises an organic solvent in a proportion by volume of about 5 to 95% by volume based on the total volume of reaction mixture.
- 5
10. A process as claimed in claim 9, where the organic solvent is selected from acetone, C₄-C₁₀-alkanes, C₄-C₁₀-alkanols, di-C₂-C₆-alkyl ethers and aromatic solvents and mixtures thereof.
- 10
11. A process as claimed in any of claims 1 to 3, where the carotenoid ester-containing reactant is incubated with the ester-cleaving enzyme in a substantially nonaqueous reaction medium.
12. A process as claimed in claim 11, where the nonaqueous reaction medium comprises at least one organic solvent selected from C₄-C₁₀-alkanes, C₄-C₁₀-alkanols, di-C₂-C₆-alkyl ethers and aromatic solvents.
- 15
13. A process as claimed in any of the preceding claims, where the enzyme is a lipase from *Candida* sp. or a cholesterol esterase from *Pseudomonas* or a mixture thereof.
- 20
14. A process as claimed in any of the preceding claims, where the reactant comprises at least one carotenoid monoester or diester of a C₁₀₋₂₄, preferably C₁₂₋₂₀ monocarboxylic acid.
- 25
15. A process as claimed in any of the preceding claims, where the reactant is derived from natural or recombinant pro- or eukaryotic microorganisms or plants or parts thereof.
- 30
16. A process as claimed in claim 15, where the reactant is obtained by extraction with the aid of an organic solvent or mixtures of organic solvents.
17. A process as claimed in any of the preceding claims, where the reaction is carried out in a plurality of stages with repeated addition of enzyme.
- 35
18. A process as claimed in any of the preceding claims, where the enzyme is employed in carrier-bound form.
19. A process as claimed in any of the preceding claims, where the reaction temperature is in the range of about 20 to 70°C.
- 40

20. A process as claimed in any of the preceding claims, where the carotenoid ester-containing reactant includes at least one carotenoid ester, at least one ketocarotenoid ester or mixtures of carotenoid esters and ketocarotenoid esters.
- 5 21. A process as claimed in any of the preceding claims, where the cleaved carotenoids and/or ketocarotenoids are isolated from the reaction medium.
22. A process as claimed in any of the preceding claims, where the ketocarotenoid ester is derived from astaxanthin.
- 10 23. The use of the carotenoids prepared as set forth in any of the preceding claims for producing human and animal food additions.

Abstract

The present invention relates to a process for the enzymatic hydrolysis of carotenoid esters, and to the use of the carotenoids prepared in this way as human and animal

5

foods.